DELIVERED BY HAND ON JULY 2, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: He et al.

Application Serial No.: Unassigned

Filed: Herewith

Title: Interleukin-1 Beta Converting Enzyme Like Apoptosis Protease-3 and 4

Attorney Docket No.: PF140C2

Art Unit: Unassigned

Examiner: Unassigned

PRELIMINARY AMENDMENT

Commissioner For Patents
Washington, D.C. 20231

Sir:

Prior to examination, applicants hereby request that the following amendment and remarks be entered into the above-identified application. Applicants submit concurrently herewith: (a) Submission of Substitute/Formal Drawings of Figures 1A-B, 2A-B, and 3A-C (7 sheets) with copies of drawings as originally filed with changes marked; (b) a Substitute Sequence Listing in both paper and computer-readable forms; and (c) in accordance with 37 C.F.R. § 1.121(c)(1)(ii), a Version of Amendments With Markings to Show Changes Made. Please amend the application as follows.

In the Specification:

On page 1, after the title, please insert

--This application is a continuation of, and claims priority under 35 U.S.C § 120 to, U.S. Patent Application No. 08/334,251, filed November 1, 1994, which is herein incorporated by reference in its entirety.--.

Please replace the paragraph beginning at page 4, line7, with the following written paragraph:

-- Figures 1A-B show the cDNA and corresponding deduced amino acid sequence of ICE-LAP-3. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the

initial methionine residue), and the standard one-letter abbreviation for amino acids is used. --

Please replace the paragraph beginning at page 4, line 12, with the following written paragraph:

-- Figures 2A-B show the cDNA and corresponding deduced amino acid sequence of ICE-LAP-4. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue). --

Please replace the paragraph beginning at page 4, line 16, with the following written paragraph:

-- Figures 3A-C show an amino acid sequence comparison between ICE-LAP-3, ICE-LAP-4, human ICE and the C. elegan cell death gene ced-3. Shaded areas represent amino acid matches between the different sequences. --

Please replace the paragraph beginning at page 4, line 20, with the following written paragraph:

-- Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1A-B and 2A-B are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%. --

Please replace the paragraph beginning at page 4, line 25, with the following written paragraph:

-- In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or for the mature polypeptide encoded by the cDNA of the clones deposited as ATCC Deposit No. 75875 encoding ICE-LAP-3, and ATCC Deposit No. 75873 encoding ICE-LAP-4, which were deposited August 25, 1994. --

Please insert the following paragraph on page 4, after line 32:

--These deposits are biological deposits with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209. Since the strains referred to are being maintained under the terms of the Budapest Treaty, they will be made available to a patent office signatory to the Budapest Treaty.--

Please replace the paragraph beginning at page 4, line 33, with the following written paragraph:

-- The polynucleotide encoding ICE-LAP-3 can be detected from human prostate, human endometrial tumor, human pancreatic tumor, human adrenal gland tumor and human tonsil. The full-length encoding ICE-LAP-3 was discovered in a cDNA library derived from human endometrial tumor. It is structurally related to the Interleukin-1 converting enzyme family. It contains an open reading frame encoding a protein of approximately 341 amino acid residues. The protein exhibits the highest degree of homology to *C. elegans* cell death gene ced-3 which is a homolog of human interleukin-1 converting enzyme, with 68 % similarity and 43 % identity over the entire amino acid sequence. It should be pointed out that the pentapeptide QACRG is conserved and is located at amino acid position 184-188. --

Please replace the paragraph beginning at page 5, line 21, with the following written paragraph:

-- The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encode the mature polypeptides may be identical to the coding sequence shown in Figures 1A-B and 2A-B or that of the deposited clones or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encode the same mature polypeptides, and derivatives thereof, as the DNA of Figures 1A-B and 2A-B or the deposited cDNA. --

Please replace the paragraph beginning at page 5, line 33, with the following written paragraph:

-- The polynucleotides which encode for the mature polypeptides of Figures 1A-B and 2A-B or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide. --

Please replace the paragraph beginning at page 6, line 10, with the following written paragraph:

-- The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides. --

Please replace the paragraph beginning at page 6, line 18, with the following written paragraph:

-- Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1A-B and 2A-B or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants. --

Please replace the paragraph beginning at page 6, line 27, with the following written paragraph:

-- As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figures 1A-B and 2A-B or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of nucleotides, which does not substantially alter the function of the encoded polypeptides. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. --

Please replace the paragraph beginning at page 7, line 11, with the following written paragraph:

-- The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1A-B and 2A-B or the deposited cDNA(s). --

Please replace the paragraph beginning at page 8, line 4, with the following written paragraph:

-- The present invention further relates to ICE-LAP-3 and 4 polypeptides which have the deduced amino acid sequence of Figures 1A-B and 2A-B or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides. --

Please replace the paragraph beginning at page 8, line 9, with the following written paragraph:

-- The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1A-B and 2A-B or that encoded by the deposited cDNA, means polypeptides which retain essentially the same biological function or activity as such polypeptides, and wherein derivatives include polypeptides with enhanced or reduced biological function. An analog includes a proprotein portion to produce active mature polypeptides. --

Please replace the paragraph beginning at page 8, line 20, with the following written paragraph:

-- The fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-C or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein. --

Please replace the paragraph beginning at page 26, line 11, with the following written paragraph:

--The DNA sequence encoding ICE-LAP-3, ATCC # 75875, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-3 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-3 gene. Additional nucleotides corresponding to ICE-LAP-3 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGCGTGCGGGGACACGGGTC 3' (SEQ ID NO:5) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5'

GTACTCTAGATCATTCACCCTGGTGGAGGAT 3' (SEQ ID NO:6) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA isisolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ration of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-3 (95% pure is eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After

incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Please replace the paragraph beginning at page 28, line 1, with the following written paragraph:

-- The DNA sequence encoding ICE-LAP-4, ATCC # 75873, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-4 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-4 gene. Additional nucleotides corresponding to ICE-LAP-4 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATC<u>GGATCC</u>ATGGAGAACACTGAAAACTCA 3' (SEQ ID NO:7) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTAC<u>TCTAGA</u>TTAGTGATAAAAATAGAGTTC 3' (SEQ ID NO:8) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-4. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform <u>E. coli</u> available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA isisolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp

(100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ration of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.^{600}) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-4 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-4 (95% pure is eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Please replace the paragraph beginning at page 30, line 7, with the following written paragraph:

-- The DNA sequence encoding for ICE-LAP-3, ATCC # 75875, was constructed by PCR on the full-length ICE-LAP-3 using two primers: the 5' primer 5' GACTATGCGTGCGGGGACACGG 3' (SEQ ID NO:9) contains the ICE-LAP-3 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-3 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGTATGGGTATTCACCCTGGTGGA GGATTTG 3' (SEQ ID NO:10) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-3 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-3 coding sequence followed by HA tag fused in frame, and a translation termination stop codonnext to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the

transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-3 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

Page 31, line 6, after "SDS-PAGE gels." delete the remainder of the page which is empty space.

Please replace the paragraph beginning at page 32, line 20, with the following written paragraph:

-- The DNA sequence encoding for ICE-LAP-4, ATCC # 75873, was constructed by PCR on the full-length ICE-LAP-4 using two primers: the 5' primer 5' ACCATGGAGAACACTGAAAAC 3' (SEQ ID NO:11) contains the ICE-LAP-4 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-4 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGGTATGGGTAGTGATAAAAATA GAGTTCTTT 3' (SEQ ID NO:12) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-4 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-4 coding sequence followed by HA tag fused in frame, and a translation termination stop codonnext to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was

transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-4, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-4 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

Please replace the sequence listing at pages 35-41 of the specification with the Substitute Sequence Listing (pages 1-6) attached hereto.

In the Figures:

Please cancel Figure 1, without prejudice, and replace it with new Figures 1A-B, submitted herewith with the formal drawings.

Please cancel Figure 2, without prejudice, and replace it with new Figures 2A-B, submitted herewith with the formal drawings.

Please cancel Figure 3, without prejudice, and replace it with new Figures 3A-C, submitted herewith with the formal drawings.

REMARKS

A substitute sequence listing and substitute Figures are submitted herewith to correct the nucleotide and amino acid sequences originally presented in the ICE-LAP 3 sequences (shown in the sequence listing as SEQ ID NOs:1 and 2 and in Figures 1 and 3). These changes do not introduce new matter because the correct sequence was inherent to the originally sequenced plasmid clone in actual possession of the Applicants before the time the captioned application was filed. The analysis needed to determine the complete and correct sequences were well within the skill of the ordinary artisan, and such analysis would not have required undue experimentation.

These same corrections to the Figures were submitted and entered in related Application No: 08/462,969 (now U.S. Patent No. 6,087,150). Attached hereto as Exhibit A is a copy of the Amendment and accompanying Declaration of Craig Rosen under 37 C.F.R. § 1.132 with Exhibits A-E thereto requesting correction of the sequence listing and figures for the aforementioned application. In this amendment, Applicants demonstrate that the corrected sequences are inherently present in the deposited material and, because Applicants have demonstrated "chain of custody" for the material originally sequenced and the resequenced material, correction of the originally presented sequence herein is not new matter. Furthermore, support for the correction of such sequencing errors can be found in the specification at page 4, lines 20 – 24 which state:

> "Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1 and 2 are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%."

The corrections to SEQ ID NO:1 submitted herein do not constitute a change of greater than 3%, hence the corrected sequence is fully supported by the specification and is not new matter.

Further, the Sequence Listing filed herewith includes sequences (SEQ ID NOs:5-12) corresponding to the primer sequences set forth in the examples of the instant application. The specification is amended as set forth above to insert the appropriate SEQ ID NOs at the appropriate locations for such primer sequences.

The specification has been amended to add the cross reference to the related application.

The specification was amended to reflect the drawing designations provided for in the formal drawings.

The specification was further amended on page 5 to correct the location of the pentapeptide QACRG in SEQ ID NO:2.

The specification was further amended on page 5 to correct the spelling of "homolog".

Accordingly, no new matter has been added herein.

Statements Under 37 C.F.R. § 1.825(a) and (b)

In accordance with 37 C.F.R. § 1.825(a), the undersigned attorney for Applicants hereby states that sequence information contained in the Substitute Sequence Listing submitted herewith is completely supported by the specification as originally filed, as detailed above, and no new matter has been introduced.

In accordance with 37 C.F.R. § 1.825(b), the undersigned attorney for Applicants hereby states that the information in the paper copy of the Substitute Sequence Listing submitted herewith is identical to the information contained in the computer readable form of the Substitute Sequence Listing submitted herewith.

SUMMARY

Applicants respectfully request entry of the amendments and remarks contained in this paper. If the Examiner believes that a telephone conference would help facilitate entry of the herein amendments, she is encouraged to call Applicants' representative at the telephone number listed below.

Respectfully submitted,

Dated: July 2, 2001

Jonathan Klein

Jonathan L. Klein (Reg. No. 41,119)
Attorney for Applicants

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Telephone: (301) 251-6015

JLK/LT/ba

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: application of: He et al.

Serial No.: 08/462,969 | Group Art Unit: 1652

Filed: June 5, 1995 | Examiner: G. Bugaisky

For: **Interleukin-1 β Converting Enzyme Like Apoptosis Protease 3 and 4** | Attorney Docket No.: **PF140P1**

AMENDMENT

Assistant Commissioner
For Patents
Washington, D.C. 20231
Sir:

This Amendment is supplemental to the amendment filed March 15, 1999. A Declaration of Craig Rosen Under 37 CFR §1.132 accompanies this Amendment. Please amend the application as follows.

In the Specification:

On page 5, line 20, please replace "hoholog" with –homolog--.

On page 5, line 24, please replace "259-263" with --184-188--.

Please replace the sequence listing with the sequence listing attached hereto.

In the Figures

Please replace Figures 1 and 3 with the substitute Figures 1 and 3 attached hereto.

In the Claims:

61. (Once amended) An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence shown as [residues 1 to 341 in]SEQ ID NO:2;

(b) the amino acid sequence shown as [residues 2 to 341 in]SEQ ID NO:2, but lacking the N-terminal methionine residue;

(c) at least 30 contiguous amino acid residues of SEQ ID NO:2;

(d) the amino acid sequence of a fragment of the polypeptide shown as residues 1 to 341 in SEQ ID NO:2 wherein said polypeptide fragment has apoptosis inducing activity; and

(e) an amino acid sequence encoded by a polynucleotide capable of hybridizing to the complement of a polynucleotide consisting of SEQ ID NO:1, when incubated together in 0.5 M $NaPO_4$, pH 7.4 and 7% SDS overnight at 65°C followed by washing twice at room temperature and twice at 60°C with 0.5X SSC, and 0.1% SDS, wherein a polypeptide consisting of such amino acid sequence retains apoptosis inducing activity.

64. (Once amended) The isolated polypeptide of claim 63 comprising the amino acid sequence shown as [residues 2 to 341 in]SEQ ID NO:2, but lacking the N-terminal methionine residue.

65. (Once amended) The isolated polypeptide of claim [64] 63 comprising the amino acid sequence shown as [residues 1 to 341 in]SEQ ID NO:2.

REMARKS

Claims 61(b), 64, and 65 were amended herein. Claims 1-60 were canceled previously. Accordingly, claims 61-77 are pending. Reconsideration of the objections and rejections made in the Official action mailed October 14, 1998 is respectfully requested in light of both the Amendment filed March 15, 1999 and the present Amendment.

I. Amendments

A substitute sequence listing and substitute Figures 1 and 3 are submitted herewith to correct nucleotide and amino acid sequence errors as originally presented in the ICE-LAP 3 sequences (shown in the sequence listing as SEQ ID NOS:1 and 2). These changes do not introduce new matter because the correct sequence was inherent to the originally sequenced plasmid clone in actual possession of the Applicants before the time the captioned application was filed.

The captioned application is directed to both polynucleotides and polypeptides which are chemical compounds. The nucleotide sequence is but an inherent property of the described polynucleotides. There is a line of chemical case law where applicants have been permitted to amend the specification to correct the formula of a chemical compound after an application's filing date, provided that it had been demonstrated that one of skill in the art would have appreciated that the applicant was in possession of the compound itself at the time of filing. The rationale is that the formula is an inherent property of the compound and thus amending the specification to correct the formula is not new matter. See *In re Nathan*, 140 U.S.P.Q. 601, 604 (C.C.P.A. 1964). *Accord Kennecott Corp. v. Kyocera Int'l, Inc.*, 5 U.S.P.Q.2d 1194, 1198 (Fed. Cir. 1987), *cert. denied*, 486 U.S. 1008 (1988) ("The disclosure in a subsequent patent application of an inherent property of a product does not deprive that product of the benefit of the earlier filing date.").

In the field of biotechnology, applicants often rely on a deposited clone, where the deposit was made prior to filing, to establish possession of nucleic acids or proteins. The focus for determining whether applicants were in possession of claimed nucleic acids or proteins has been determined, at least in part, by considering whether the applicant has: (1) established that one skilled in the art in possession of the deposited clone would have been aware of both the

DNA sequence and the encoded amino acid sequence, or would be able to determine these sequences without undue experimentation, (2) established that the DNA and amino acid sequences are described in a manner such that one skilled in the art could distinguish them from other sequences, and (3) resequenced a clone which is identical to that of the deposit and established a "chain of custody" for this clone. See e.g., *Ex parte Maizel*, 27 U.S.P.Q.2d 1662, 1669-1670 (B.P.A.I. 1992).

Submitted herewith is a Declaration of Craig Rosen Under 37 CFR §1.132 (the "Rosen Declaration") which describes certain events involving the characterization of the nucleotide sequence of cDNA clone HE2CA82 which encodes ICE-LAP 3. The HE2CA82 cDNA clone was deposited with the American Type Culture Collection (ATCC) on August 25, 1994; i.e., prior to the filing date of parent application US Serial No. 08/334,251 (filed November 1, 1994). Exhibit A of the Rosen Declaration is a copy of the contract for ATCC Deposit No. 75875. The Examiner will note that the present specification states on page 5, lines 7 and 8, that ATCC Deposit No. 75875 contains the cDNA which encodes ICE-LAP 3.

The corrected nucleic acid and deduced amino acid sequences (shown in replacement Figures 1 and 3 submitted herewith) were determined by reanalyzing cDNA clone HE2CA82 (ATCC Accession No. 75875); i.e., the same cDNA clone from which the originally presented sequences were determined (see paragraph 4 of the Rosen Declaration). The corrected sequence information was published by the present inventors in a peer-reviewed scientific journal article (see Exhibit B of the Rosen Declaration).

The analysis needed to determine the complete and correct sequences of cDNA clone HE2CA82 were well within the skill of the ordinary artisan as of the filing date of the parent application US Serial No. 08/334,251 (filed November 1, 1994), and such analysis would not have required undue experimentation (see paragraph 5 of the Rosen Declaration).

Exhibit D of the Rosen Declaration shows the four (4) nucleotide differences between the original sequence ("PF140" in the Exhibit) and the corrected sequence ("Duan" in the Exhibit).

In summary, because Applicants have demonstrated that the corrected sequences are inherently present in the deposited material, and because Applicants have demonstrated"chain of custody" for the material originally sequenced and the resequenced material, correction of the orginally presented sequence information herein is not new matter.

The specification was amended on page 5 to correct the location of the pentapeptide QACRG in SEQ ID NO:2.

The specification was further amended on page 5 to correct the spelling of "homolog".

Claims 61, 64, and 65 were amended herein. Support for the amended claims can be found in the specification and claims as originally filed. In particular, support for claims 61(b) and 64 which recite a polypeptide "lacking an N-terminal methionine residue" can be found in the specification, for example, on page 17, lines 32 and 33.

Accordingly, no new matter has been added herein.

VII. Summary

Applicants believe that the application is in condition for allowance and a notice to that effect is earnestly solicited. If the Examiner believes that a telephone conference would help to facilitate prosecution of the present application she is encouraged to call Applicants' representative at the telephone number listed below.

Respectfully submitted,

Dated: 3/22/99

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AAB/mbp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: He et al.

Application Serial No.: Unassigned

Filed: Herewith

Title: Interleukin-1 Beta Converting Enzyme Like Apoptosis Protease-3 and 4

Attorney Docket No.: PF140C2

Art Unit: Unassigned

Examiner: Unassigned

VERSION OF AMENDMENTS WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 4, line7, has been amended as follows:

Figures 1A-B shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-3. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue), and the standard one-letter abbreviation for amino acids is used.

Paragraph beginning at page 4, line 12, has been amended as follows:

Figures 2A-B shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-4. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue).

Paragraph beginning at page 4, line 16, has been amended as follows:

Figures 3A-C shows an amino acid sequence comparison between ICE-LAP-3, ICE-LAP-4, human ICE and the C. elegans cell death gene ced-3. Shaded areas represent amino acid matches between the different sequences.

Paragraph beginning at page 4, line 20, has been amended as follows:

Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1A-B and 2A-B are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%.

Paragraph beginning at page 4, line 25, has been amended as follows:

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or for the mature polypeptide encoded by the cDNA of the clones deposited as ATCC Deposit No. 75875 encoding ICE-LAP-3, and ATCC Deposit No. 75873 encoding ICE-LAP-4, which were deposited August 25, 1994.

Paragraph beginning at page 4, line 33, has been amended as follows:

The polynucleotide encoding ICE-LAP-3 can be detected from human prostate, human endometrial tumor, human pancreatic tumor, human adrenal gland tumor and human tonsil. The full-length encoding ICE-LAP-3 was discovered in a cDNA library derived from human endometrial tumor. It is structurally related to the Interleukin-1 converting enzyme family. It contains an open reading frame encoding a protein of approximately 341 amino acid residues. The protein exhibits the highest degree of homology to *C. elegans* cell death gene ced-3 which is a ~~hoholog~~ homolog of human interleukin-1 converting enzyme, with 68 % similarity and 43 % identity over the entire amino acid sequence. It should be pointed out that the pentapeptide QACRG is conserved and is located at amino acid position ~~259-263~~ 184-188.

Paragraph beginning at page 5, line 21, has been amended as follows:

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encode the mature polypeptides may be identical to the coding sequence shown in Figures 1A-B and 2A-B or that of the deposited clones or may be a different coding sequence which coding sequence, as a

result of the redundancy or degeneracy of the genetic code, encode the same mature polypeptides, and derivatives thereof, as the DNA of Figures 1A-B and 2A-B or the deposited cDNA.

Paragraph beginning at page 5, line 34, has been amended as follows:

The polynucleotides which encode for the mature polypeptides of Figures 1A-B and 2A-B or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Paragraph beginning at page 6, line 10, has been amended as follows:

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides.

Paragraph beginning at page 6, line 18, has been amended as follows:

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1A-B and 2A-B or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

Paragraph beginning at page 6, line 27, has been amended as follows:

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in

Figures 1A-B and 2A-B or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of nucleotides, which does not substantially alter the function of the encoded polypeptides. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues.

Paragraph beginning at page 7, line 11, has been amended as follows:

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1A-B and 2A-B or the deposited cDNA(s).

Paragraph beginning at page 8, line 4, has been amended as follows:

The present invention further relates to ICE-LAP-3 and 4 polypeptides which have the deduced amino acid sequence of Figures 1A-B and 2A-B or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides.

Paragraph beginning at page 8, line 9, has been amended as follows:

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1A-B and 2A-B or that encoded by the deposited cDNA, means polypeptides which retain essentially the same biological function or activity as such polypeptides, and wherein derivatives include

polypeptides with enhanced or reduced biological function. An analog includes a proprotein portion to produce active mature polypeptides.

Paragraph beginning at page 8, line 20, has been amended as follows:

The fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-C or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Paragraph beginning at page 26, line 11, has been amended as follows:

--The DNA sequence encoding ICE-LAP-3, ATCC # 75875, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-3 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-3 gene. Additional nucleotides corresponding to ICE-LAP-3 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGCGTGCGGGGACACGGGTC 3' (SEQ ID NO:5) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATCATTCACCCTGGTGGAGGAT 3' (SEQ ID NO:6) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable

promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA isisolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnig t (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ration of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-3 (95% pure is eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.—

Paragraph beginning at page 28, line 1, has been amended as follows:

-- The DNA sequence encoding ICE-LAP-4, ATCC # 75873, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-4 protein (minus the signal peptide

sequence) and the vector sequences 3' to the ICE-LAP-4 gene. Additional nucleotides corresponding to ICE-LAP-4 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGGAGAACACTGAAAACTCA 3' (SEQ ID NO:7) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATTAGTGATAAAAATAGAGTTC 3' (SEQ ID NO:8) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-4. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA isisolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ration of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-4 is purified from

this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-4 (95% pure is eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Paragraph beginning at page 30, line 7, has been amended as follows:

-- The DNA sequence encoding for ICE-LAP-3, ATCC # 75875, was constructed by PCR on the full-length ICE-LAP-3 using two primers: the 5' primer 5' GACTATGCGTGCGGGACACGG 3' (SEQ ID NO:9) contains the ICE-LAP-3 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-3 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGTATGGGTATTCACCCTGGTGGAGGATTTG 3' (SEQ ID NO:10) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-3 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-3 coding sequence followed by HA tag fused in frame, and a translation termination stop codonnext to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-3 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory

Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

Paragraph beginning at page 32, line 20, has been amended as follows:

-- The DNA sequence encoding for ICE-LAP-4, ATCC # 75873, was constructed by PCR on the full-length ICE-LAP-4 using two primers: the 5' primer 5' ACCATGGAGAACACTGAAAAC 3' (SEQ ID NO:11) contains the ICE-LAP-4 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-4 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGGTATGGGTAGTGATAAAAATA GAGTTCTTT 3' (SEQ ID NO:12) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-4 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-4 coding sequence followed by HA tag fused in frame, and a translation termination stop codonnext to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-4, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-4 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with

detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

1 GCACGAGAAACTTTGCTGTGCGCGTTCTCCCGCGCGCGGGCTCAACTTTGTAGAGCGAGG

61 GGCCAACTTGGCAGAGCGCGCGGCCAGCTTTGCAGAGAGCGCCCTCCAGGGACTATGCGT

1 ~~M R~~

121 GCGGGGACACGGGTCGCTTTGGGCTCTTCCACCCCTGCGGAGCGCACTACCCCGAGCCAG

3 ~~A G T R V A L G S S T P A E R T T P S Q~~

181 GGGCGGTGCAAGCCCCGCCCGGCCCTACCCAGGGCGGCTCCTCCCTCCGCAGCGCCGAGA

23 ~~G R C K P R P A L P R A A P P S A A P R~~

241 CTTTTAGTTTCGCTTTCGCTAAAGGGGCCCCAGACCCTTGCTGCGGAGCGACGGAGAGAG

43 ~~L L V S L S L K G P Q T L A A E R R R E~~

301 ACTGTGCCAGTCCCAGCCGCCCTACCGCCGTGGAACGATGGCAGATGATTCAGGGCTGT

63 ~~T V P V P A A L P P W E R~~ ~~W~~M ~~Q~~A ~~M~~D ~~I~~D Q G C

361 ATTGAAGAGCAGGGGGTTGAGGATTCAGCAAATGAAGATTCAGTGGATGCTAAGCCAGAC

83 I E E Q G V E D S A N E D S V D A K P D

421 CGGTCCTCGTTTGTACCGTCCCTCTTCAGTAAGAAGAAGAAAAATGTCACCATGCGATCC

103 R S S F V P S L F S K K K K N V T M R S

481 ATCAAGACCACCCGGGACCGAGTGCCTACATATCAGTACAACATGAATTTTGAAAAGCTG

123 I K T T R D R V P T Y Q Y N M N F E K L

541 GGCAAATGCATCATAATAAACAACAAGAACTTTGATAAAGTGACAGGTATGGGCGTTCGA

143 G K C I I I N N K N F D K V T G M G V R

601 AACGGAACAGACAAAGATGCCGAGGCGCTCTTCAAGTGCTTCCGAAGCCTGGGTTTTGAC

163 N G T D K D A E A L F K C F R S L G F D

661 GTGATTGTCTATAATGACTGCTCTTGTGCCAAGATGCAAGATCTGCTTAAAAAAGCTTCT

183 V I V Y N D C S C A K M Q D L L K K A S

721 GAAGAGGACCATACAAATGCCGCCTGCTTCGCCTGCATCCTCTTAAGCCATGGAGAAGAA

203 E E D H T N A A C F A C I L L S H G E E

781 AATGTAATTTATGGGAAAGATGGTGTCACACCAATAAAGGATTTGACAGCCCACTTTAGG

223 N V I Y G K D G V T P I K D L T A H F R

841 GGGGATAGATGCAAAACCCTTTTAGAGAAACCCAAACTCTTCTTCATTCAGGCTTGCCGA

243 G D R C K T L L E K P K L F F I Q A C R

901 GGGACCGAGCTTGATGATGGCCATCCAGGCCGACTCGGGGCCCATCAATGACACAGATGCT

263 G T E L D D ~~G~~A I Q A D S G P I N D T D A

961 AATCCTCGATACAAGATCCCAGTGGAAGCTGACTTCCTCTTCGCCTATTCCACGGTTCCA

283 N P R Y K I P V E A D F L F A Y S T V P

1021 GGCTATTACTCGTGGAGGAGCCCAGGAAGAGGCTCCTGGTTTGTGCAAGCCCTCTGCTCC

303 G Y Y S W R S P G R G S W F V Q A L C S

1081 ATCCTGGAGGAGCACGGAAAAGACCTGGAAATCATGCAAATCCTCCACCAGGGTGAATGA

323 I L E E H G K D L E I M Q I L ~~H~~T ~~Q~~R ~~G~~V ~~F~~N ~~I~~D

1141 CAGAGTTGCCAGGCACTTTGAGTCTCAGTCTGATGACCCACACTTCCATGAGAAGAAGCA

R V A R H F E S Q S D D P H F H E K K Q

1201 GATCCCCTGTGTGGTCTCCATGCTCACCAAGGAACTCTACTTCAGTCAATAGCCATATCA

I P C V V S M L T K E L Y F S Q

1261 GGGGTACATTCTAGCTGAGAAGCAATGGGTCACTCATTAATGAATCACATTTTTTATGC

1321 TCTTGAAATATTCAGAAATTCTCCAGGATTTTAATTTCAGGAAAATGTATT

Figure 1

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1      GCACGAGCGGATGGGTGCTATTGTGAGGCGGTTGTAGAAGAGTTTCGTGAGTGCTCGCAG

61     CTCATACCTGTGGCTGTGTATCCGTGGCCACAGCTGGTTGGCGTCGCCTTGAAATCCCAG

121    GCCGTGAGGAGTTAGCGAGCCCTGCTCACACTCGGCGCTCTGGTTTTCGGTGGTGTGCC

181    CTGCACCTGCCTCTTCCCGCATTCTCATTAATAAAGGTATCCATGGAGAACACTGAAAAC
1                                                    M  E  N  T  E  N
241    TCAGTGGATTCAAAATCCATTAAAAATTTGGAACCAAAGATCATACATGGAAGCGAATCA
7      S  V  D  S  K  S  I  K  N  L  E  P  K  I  I  H  G  S  E  S
301    ATGGACTCTGGAATATCCCTGGACAACAGTTATAAAATGGATTATCCTGAGATGGGTTTA
27     M  D  S  G  I  S  L  D  N  S  Y  K  M  D  Y  P  E  M  G  L
361    TGTATAATAATTAATAATAAGAATTTTCATAAAAGCACTGGAATGACATCTCGGTCTGGT
47     C  I  I  I  N  N  K  N  F  H  K  S  T  G  M  T  S  R  S  G
421    ACAGATGTCGATGCAGCAAACCTCAGGGAAACATTCAGAAACTTGAAATATGAAGTCAGG
67     T  D  V  D  A  A  N  L  R  E  T  F  R  N  L  K  Y  E  V  R
481    AATAAAAATGATCTTACACGTGAAGAAATTGTGGAATTGATGCGTGATGTTTCTAAAGAA
87     N  K  N  D  L  T  R  E  E  I  V  E  L  M  R  D  V  S  K  E
541    GATCACAGCAAAAGGAGCAGTTTTGTTTGTGTGCTTCTGAGCCATGGTGAAGAAGGAATA
107    D  H  S  K  R  S  S  F  V  C  V  L  L  S  H  G  E  E  G  I
601    ATTTTTGGAACAAATGGACCTGTTGACCTGAAAAAAATAACAAACTTTTTCAGAGGGGAT
127    I  F  G  T  N  G  P  V  D  L  K  K  I  T  N  F  F  R  G  D
661    CGTTGTAGAAGTCTAACTGGAAAACCCAAACTTTTCATTATTCAGGCCTGCCGTGGTACA
147    R  C  R  S  L  T  G  K  P  K  L  F  I  I  Q  A  C  R  G  T
721    GAACTGGACTGTGGCATTGAGACAGACAGTGGTGTTGATGATGACATGGCGTGTCATAAA
167    E  L  D  C  G  I  E  T  D  S  G  V  D  D  D  M  A  C  H  K
781    ATACCAGTGGAGGCCGACTTCTTGTATGCATACTCCACAGCACCTGGTTATTATTCTTGG
187    I  P  V  E  A  D  F  L  Y  A  Y  S  T  A  P  G  Y  Y  S  W
841    CGAAATTCAAAGGATGGCTCCTGGTTCATCCAGTCGCTTTGTGCCATGCTGAAACAGTAT
207    R  N  S  K  D  G  S  W  F  I  Q  S  L  C  A  M  L  K  Q  Y
901    GCCGACAAGCTTGAATTTATGCACATTCTTACCCGGGTTAACCGAAAGGTGGCAACAGAA
227    A  D  K  L  E  F  M  H  I  L  T  R  V  N  R  K  V  A  T  E
961    TTTGAGTCCTTTTCCTTTGACGCTACTTTTCATGCAAAGAAACAGATTCCATGTATTGTT
247    F  E  S  F  S  F  D  A  T  F  H  A  K  K  Q  I  P  C  I  V
1021   TCCATGCTCACAAAAGAACTCTATTTTTATCACTAAAGAAATGGTTGGTTGGTGGTTTTT
267    S  M  L  T  K  E  L  Y  F  Y  H  *
1081   TTTAGTTTGTATGCCAAGTGAGAAGATGGTATATTTGGGTACTGTATTTCCCTCTCATTG

1141   GGGACCTACTCTCATGCTG
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Figure 2

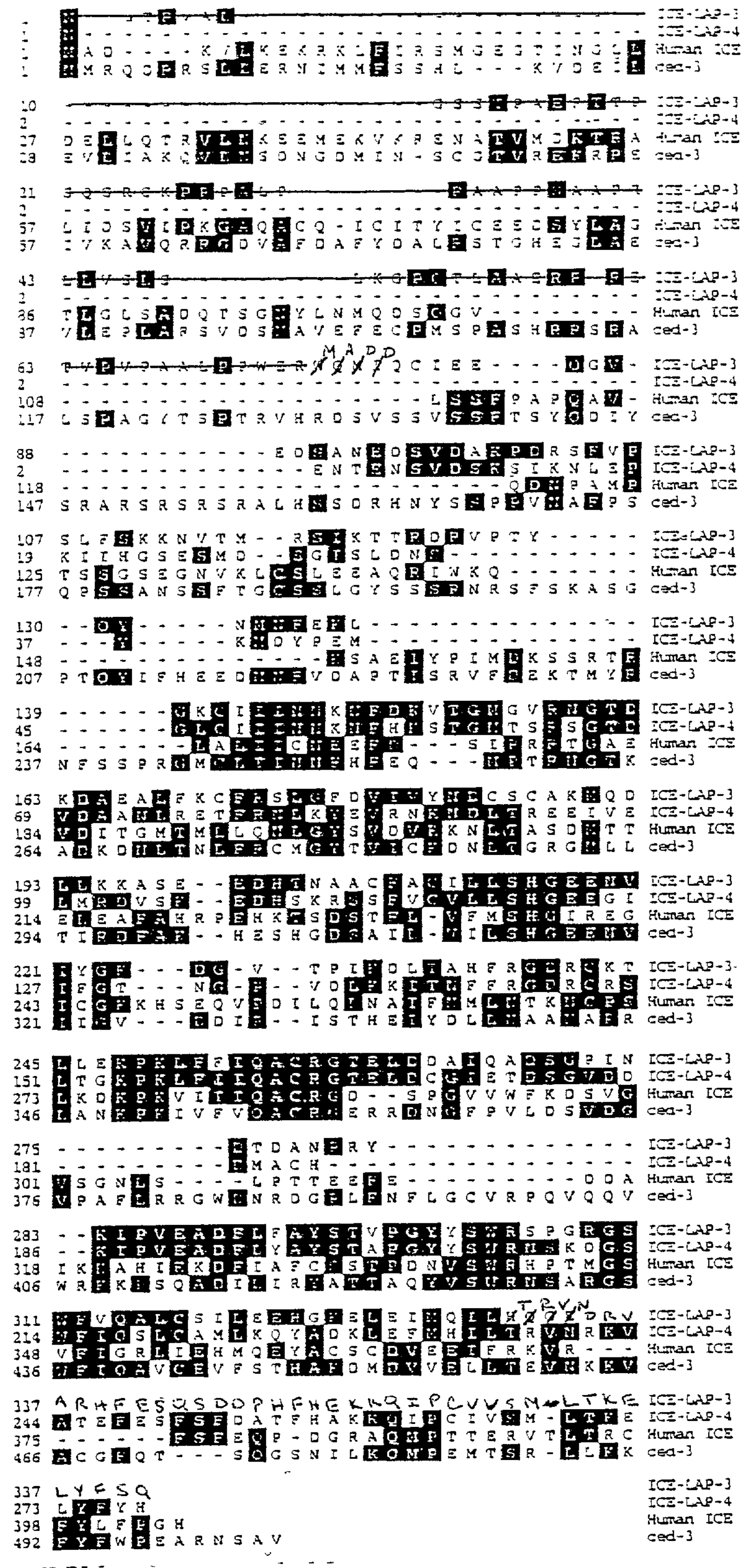

Figure 3